Marine Pollution Bulletin 141 (2019) 70–78

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Effects of ocean warming and acidification on fertilization success and early larval development in the green sea urchin *Lytechinus variegatus*

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ARTICLE INFO

Keywords:
Acidification
Climate change
Echinoids
Fertilization
Larval growth
Larval development

ABSTRACT

Ocean acidification and warming are predicted to affect the early life of many marine organisms, but their effects can be synergistic or antagonistic. This study assessed the combined effects of near-future (2100) ocean acidification (pH 7.8) and warming (+3 °C) on the fertilization, larval development and growth of the green sea urchin, *Lytechinus variegatus*, common in tropical reefs of Florida and the Caribbean. Acidification had no effect on fertilization, but delayed larval development, stunted growth, and increased asymmetry. Warming decreased fertilization success when the sperm:egg ratio was higher (1847:1), accelerated larval development, but had no effect on growth. When exposed to both acidification and warming, fertilization rates decreased, larval development accelerated (due to increased respiration/metabolism), but larvae were smaller and more asymmetric, meaning acidification and warming had additive effects. Thus, climate change is expected to decrease the abundance of this important herbivore, exacerbating macroalgal growth and dominance on coral reefs.

1. Introduction

Increased levels of carbon dioxide and other greenhouse gases in the atmosphere are causing changes to global climate (IPCC, 2014). Burning fossil fuels has led to the exponential increase in carbon dioxide in the atmosphere (Canadell et al., 2007). Higher levels of carbon dioxide trap more heat in the atmosphere, resulting in increased global temperatures. The ocean absorbs this excess heat in addition to atmospheric carbon dioxide, resulting in ocean acidification and warming (OAW) (Revelle and Suess, 1957; Takahashi et al., 1997) and the reduction of carbonate ion concentrations (Zeebe and Wolf-Gladrow, 2001; Caldeira and Wickett, 2003; Orr et al., 2005; Meehl et al., 2007; Feely et al., 2009). Global ocean temperatures have risen ca. 0.17 °C per decade, and are predicted to be 2–4 °C higher on average by the end of the century (IPCC, 2014). Dissolved carbon dioxide also reacts with water to form carbonic acid ($CO_2 + H_2O \rightarrow H_2CO_3$). Carbonic acid then dissociates into hydrogen ions and bicarbonate ($H_2CO_3 \rightarrow H^+ + HCO_3^-$). The increase in hydrogen ion concentration reduces seawater pH, thus acidifying the ocean. Ocean pH is projected to drop 0.1 to 0.4 units by 2100 (Orr et al., 2005; Meehl et al., 2007; IPCC, 2014). In addition to acidification, the hydrogen ions bind to carbonate ions to create more bicarbonate ($H^+ + CO_3^{2-} \rightarrow HCO_3^-$). As carbonate ions become depleted, seawater becomes undersaturated in aragonite and calcite, which are calcium carbonate minerals that many marine organisms require to build their shells and skeletons (Dupont et al., 2010a; Hofmann et al., 2010; Kroeker et al., 2013).

Ocean warming and acidification affect the survival and growth of numerous species, including during critical life stages for recruitment and replenishment of populations, such as fertilization and larval development. Fertilization in species with external fertilization can be more vulnerable to warming and acidification (Byrne and Przeslawski, 2013, Przeslawski et al., 2015). Reduced pH can reduce sperm motility (Morita et al., 2010), and consequently fertilization success (Havenhand et al., 2008; Moulin et al., 2011; Barton et al., 2012; Albright and Mason, 2013; Kapsenberg et al., 2017). Larval survival and development can also be drastically affected by temperature and pH variations beyond the tolerance range of a species (Pechenik, 1987; Chen and Chen, 1992; Hofmann et al., 2010). Warmer temperatures often hasten larval development and growth rates, however, when too high, can arrest development and become lethal (O'Connor et al., 2007; Byrne and Przeslawski, 2013; Figueiredo et al., 2014). Differences in susceptibility to warming likely depend on the magnitude of temperature increase tested relative to the natural thermal range of the species, with tropical species (i.e., that evolved within a narrow temperature range) being generally more sensitive (Pörtner and Knust, 2007; Tewksbury et al., 2008). Ocean acidification can negatively affect the development and growth of larvae, particularly larvae with external calcified structures (Orr et al., 2005), such as oyster and mussel veligers

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https://doi.org/10.1016/j.marpolbul.2019.02.018
Received 22 August 2018; Received in revised form 3 February 2019; Accepted 10 February 2019
Available online 18 February 2019
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(Kurihara, 2008; Watson et al., 2009; Barton et al., 2012). The direct exposure to lower pH and low concentrations of calcium carbonate minerals prevents the larvae of these species to build their shells, delaying development, reducing growth, and increasing abnormalities and mortality. Larvae without calcifying structures, such as coral planula (Chua et al., 2013) and lecithotrophic sea star larva (Dupont et al., 2010b; Nguyen et al., 2012), or with internal skeletons, such as sea urchin echinopluteus (Kurihara, 2008; Sheppard Brennand et al., 2010; Byrne et al., 2013a; Byrne et al., 2013b) are typically less sensitive to near-future acidification. Larvae can, up to a certain point, maintain their internal pH at higher levels than in the environment and thus enable intracellular calcification (Stumpp et al., 2012); however this has energetic costs (Jager et al., 2016). Allocating energy to maintain homeostasis diverts energy from development and growth (Stumpp et al., 2012; Jager et al., 2016). Thus, even larvae without external calcified structures can experience some delay in larval development and abnormalities under acidification; however, these effects are commonly only present or more pronounced at higher levels of acidification, typically beyond projections for 2100 (Byrne, 2011; Byrne, 2012).

The impacts of ocean warming and acidification may be additive, synergistic or even antagonistic, with one stressor potentially prevailing over the other. Studies assessing the combined effects of ocean warming and acidification indicate that most species are more vulnerable to the effects of ocean acidification when the water was warmest (Parker et al., 2009; Anlauf et al., 2011; Rodolfo-Metalpa et al., 2011). Reductions in fertilization success and alterations to larval duration lead to a reduction in larval production, changes in dispersal patterns and gene flow between populations. Ultimately these undermine recruitment, population replenishment, and therefore species persistence (Munday et al., 2008; Munday et al., 2009), potentially leading to significant changes in the community structure of ecosystems and their socioeconomic value. To better predict the impacts of climate change in marine ecosystems, it is critical to assess the combined effects of ocean acidification and warming on species with key ecosystem functions (e.g. herbivory or habitat construction), particularly those in which all life stages are expected to be affected, such as sea urchins.

Sea urchins control algal growth on a wide range of ecosystems, from macroalgal beds to kelp forests (Elner and Vadas Sr., 1990), rocky reefs (Andrew, 1993) and coral reefs (Hughes et al., 1987). Sea urchins are gonochoric broadcast spawners that release eggs and sperm into the water column (James and Siikavuopio, 2011). Fertilization success is dependent on sperm concentration and egg density. Low sperm concentration reduces fertilization success due to lower chances of gamete encounter, whereas abundant sperm can result in polyspermy (Levitan, 2004; Levitan and Ferrell, 2006). When eggs are abundant, egg competition dictates that larger eggs will more likely be fertilized than smaller eggs because the sperm more easily encounters them. With a low egg density, the eggs will again be at risk of polyspermy because there will be sperm competition over the low number of eggs (Levitan, 1993, 2004). Activation of sperm occurs by intracellular ion concentration, where the ATPase activity of sperm depends upon the pH of the medium (Christen et al., 1982, 1983). As pH increases, ATPase activity increases, however, dynein ATPase is inactive below a pH of 7.3, thus inhibiting sperm activation (Christen et al., 1982, 1983).

Despite being keystone species in many ecosystems, the combined effects of ocean warming and acidification on the fertilization and/or larval development on sea urchins have only been assessed in four species, all from southeast Australia (temperate region). Existent studies did not detect deleterious effects on fertilization (Byrne et al., 2010a), but show that warming and acidification have additive or antagonistic effects on larvae, with warming reducing the negative effects of acidification, but with the prevailing effect differing between species (Byrne and Przeslawski, 2013). Despite their expected lower tolerance to changes in climate, sea urchins inhabiting tropical coral reefs and adjacent sea grass beds are poorly studied. This study evaluated for the first time the combined effects of ocean acidification and warming (OAW) on the fertilization success, larval growth and development of a tropical sea urchin. The species *Lytechinus variegatus* is a common species on the reefs and seagrass beds of the Florida Reef Tract and the Caribbean Sea, where it plays an important role controlling macroalgae. We also assessed the how the respiration rate (a proxy for metabolism) of the larvae of this species was affected under warm conditions, as it helped explained some of the results of the previous experiments. Determining the effects of OAW on the fertilization success and larval development of tropical reef-dwelling sea urchins will help predict the future of coral reef ecosystems, particularly the processes that may facilitate phase-shifts from coral to macroalgal-dominance.

2. Methods

2.1. Species description and specimen collection

The green sea urchin, *Lytechinus variegatus*, is commonly found in shallow waters, typically on seagrass beds, rocky substrates or reefs, throughout the Western Atlantic and Caribbean (Watts et al., 2001; Schultz, 2006). This species feeds on algae and seagrasses, and is sexually mature at 40 mm test diameter, but adults can reach 110 mm across (Moore et al., 1963). Both fertilization and larval development of *L. variegatus* have not been previously studied under OAW conditions.

Specimens were collected during high tide at Blue Heron Bridge in Riviera Beach, Florida (26°46′58.3″N, 80°02′37.0″W), during shore dives using SCUBA. For each trial, 50 individuals (> 40 mm across) were collected to ensure that enough mature males and females were available for successful fertilization. Upon collection, individual sea urchins were placed in mesh bags underwater, and then placed into a cooler with seawater inshore to be transported to Nova Southeastern University's Guy Harvey Oceanographic Center (NSU GHOC). Temperature, salinity, and pH were measured onsite at Blue Heron Bridge. At NSU GHOC, the sea urchins were placed into a 379-L recirculating tank and maintained at conditions that mimicked the natural environment (28 °C, 35 ppt, pH 8.1) and fed macroalgae (*Caulerpa* sp. and turf algae).

2.2. Experiment 1: effect of ocean warming and acidification on fertilization

Spawning and fertilization followed methods described by Levitan et al. (1991, 1992) and Levitan (2000). Three samples from the egg (1 mL) and sperm (0.1 mL) stocks were collected and the number of gametes counted to estimate gamete density in each stock solution, and the final sperm:egg ratio. The egg and sperm stocks were then divided evenly among the 500-mL mason jars (three per treatment) containing 100 mL of filtered seawater with experimental pH and temperature adjusted to represent each treatment. This experiment was conducted in two trials. In the first trial (hereafter Trial 1), there were four treatments: 1) 28 °C and pH 8.1 (control-ambient), 2) 28 °C and pH 7.8 (ocean acidification treatment - OA), 3) 31 °C and pH 8.1 (ocean warming treatment - OW), and 4) 31 °C and pH 7.8 (ocean warming and acidification treatment - OAW). All non-control treatments represent conditions projected for 2100 (IPCC, 2014). In the second trial (hereafter Trial 2), we used the same exact methodology except that an extra temperature (33 °C) was included in an attempt to observe a further trend in the effects of elevated temperature on fertilization success. Thus, aside from the previously described treatments 1–4, there was treatment 5) 33 °C and ambient pH 8.1. Mason jars were placed in a water bath equipped with a heater and controller that maintained experimental temperatures within 1 °C of the desired temperature. The pH was maintained within 0.05 units of the desired pH by bubbling CO_2 into the mason jars using an American Marine Pinpoint® pH probe and controller connected to a CO_2 tank. Whenever the pH probe (which did measurements continuously) measured a pH level 0.05 units above the target pH (7.8), it communicated with the respective pH controller to

bubble CO_2 in the water; once the pH probe measured a pH level 0.05 units below the target pH, it communicated with the respective controller, and CO_2 bubbling would cease. After an hour of exposure, two 1 mL samples of eggs per replicate jar were observed under a microscope to check for the presence of the fertilization envelope, and the ratio of fertilized eggs to total number of eggs was recorded. The larvae resulting from Trial 1 were used in Experiment 2, while the larvae resulting from Trial 2 were used in Experiment 3.

2.3. Experiment 2: effect of ocean warming and acidification on larval growth and development

The embryos from the first fertilization trial were used to test the effect of warming and acidification on larval development and growth. Specifically, embryos from each fertilization experimental treatment were gently poured into a larval culture tank (static) with the same pH and temperature conditions. Embryos were reared indoors throughout their early larval development stages in 11.35 L plastic cylindrical tanks at: 1) 28 °C and pH 8.1 (control-ambient), 2) 28 °C and pH 7.8 (OA), 3) 31 °C and pH 8.1 (OW), and 4) 31 °C and pH 7.8 (OAW). Each treatment was replicated in three tanks. Each tank was subject to constant moderate aeration using a 120-V Danner air pump to maintain a constant water movement and keep larvae in suspension. The temperature of each tank was maintained within 1 °C of the desired temperature using heaters connected to temperature controllers. Room temperature was controlled by setting the air conditioning below the tested temperature. All tanks were supplied with 1 µm filtered and UV-sterilized seawater. Tanks subject to reduced pH (7.8) each had an American Marine Pinpoint® pH probe and controller connected to a CO_2 tank to maintain the pH within 0.05 units of the desired using the pH. Specifically, the whenever the pH probe inside each tank (which did measurements continuously) measured a pH level 0.05 units above the target pH (7.8), it communicated with the respective pH controller to bubble CO_2 in that tank; once the pH probe measured a pH level 0.05 units below the target pH, it communicated with the respective controller, and CO_2 bubbling would cease. Temperature, salinity and pH from each tank were measured daily to ensure desired conditions and accurate measurement by in-tank pH probes. All pH probes were calibrated weekly with VWR Analytical BDH® buffers. Salinity was maintained at 35 by adding reverse osmosis water as needed. Ammonia was checked every day. Elevated levels of ammonia (> 0.10 ppm) indicating poor water quality were recorded on day 3, thus, water changes were performed on day 3 and every other day thereafter by gentle filtration using a 25 µm mesh filter to prevent the loss of larvae. There was no evidence of damage to larvae due to filtration. The larvae were provided with a minimum of 710 particles (10–50 µm) per larva of Zeigler Feeds EZ Larva daily beginning on day 2 post-fertilization (George et al., 2004), and thus should be considered fed ad libitum.

Larvae were sampled daily from day 1 post-fertilization until day 5, and then every other day until no larvae remained (day 13). Each sample was placed onto a Sedgewick-Rafter counting cell microscope slide (1 mL) and development stages of all larvae were recorded. A minimum of ten individuals from each treatment were randomly selected and photographed under the microscope using a LC20 Olympus Camera. The photos were analyzed in the program CellSens to determine the developmental stage (Mazur and Miller, 1971; McEdward and Herrera, 1999) and measure total body length, rod length, and body width (Fig. 1, following Sheppard Brennand et al., 2010; Stumpp et al., 2011; Byrne et al., 2013a). Total body length was measured from the base of the body to a line drawn between the rod tips so that the intersection was perpendicular (Fig. 1). Length of both lateral rods was measured from the rod tip to the center of the bottom of the larva (Fig. 1). Body width was measured across the body immediately above the gut (Dorey et al., 2013; Fig. 1). Asymmetry of the two rod lengths of each larva was determined as (modified from Lamare et al., 2016):

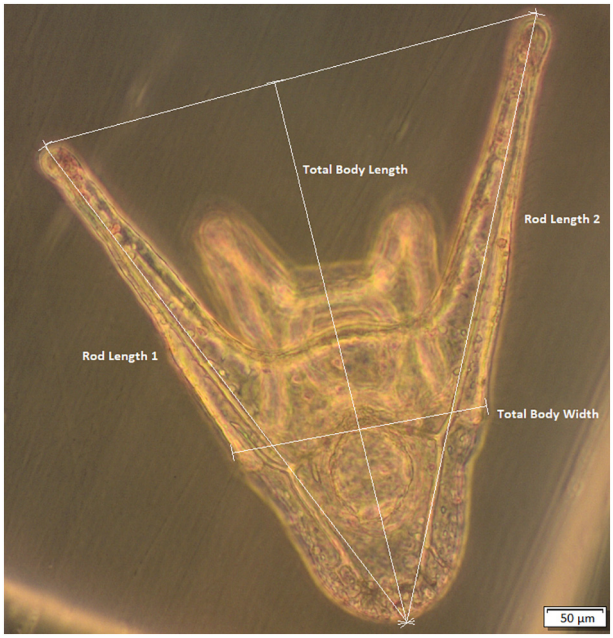

Fig. 1. Echinopluteus larval growth measurements: total body length, length of each rod and total body width.

$$\frac{|\text{rod length 1(mm)} - \text{rod length 2(mm)}|}{\text{maximum rod length(mm)}}$$

2.4. Experiment 3: effect of ocean warming on larval respiration

Evaluation of respiration under the different experimental temperatures (28 and 31 °C, 6 replicates per treatment) followed methods adapted from Edmunds et al. (2011). Two days after fertilization, the three replicates of embryos/larvae originally from Trial 2 of Experiment 1 kept at ambient conditions (28 °C, pH 8.1) were pooled. Two 200 mL samples were taken from this mixture and placed in glass jars located in two water baths equipped with heaters and controllers, one at 28 °C and the other at 31 °C. To minimize potential measurements errors, including oxygen consumption by the oxygen probe, two glass jars containing only seawater were also kept in the 28 and 31 °C water baths. All jars were aerated in order to saturate the samples with oxygen. All samples were given 2 h to acclimate to the temperature of the water bath. After the acclimation period, three 1 mL subsamples from each jar containing larvae, and one 1 mL sample from each jar containing only saltwater (blank), were put into 1 mL Wheaton vials, each with an oxygen sensor spot (SP-PSt3-NAU, PreSens) glued to its inside wall prior to the start of the experiment. Each vial was topped off with saltwater and sealed with Parafilm™ to ensure that no air was contained within.

To measure initial oxygen concentration, a polymer optical cable (POF, PreSens) was used to scan the oxygen sensor spot in the vial. The POF measures oxygen saturation (% O_2) by transferring a light from the cable to the sensor and back to the Fibox 4® meter (PreSens). The oxygen concentration of each vial was then recorded, and each vial was returned to the water bath at the designated temperatures. The vials were swirled every 5 min to keep larvae from settling and to ensure a homogenous sample. After 1 h, all vials were removed to re-measure oxygen concentration.

Oxygen consumption was calculated by subtracting the final from the initial oxygen concentration. These measurements were adjusted by subtracting the oxygen consumption in the blank sample, thereby accounting for the amount of oxygen lost to the oxygen probe or diffusion of oxygen within the vial. Then, oxygen consumed was divided by the total number of larvae per vial (counted in a Sedgewick-Rafter counting

cell microscope slide) and experimental duration to determine how much oxygen was consumed per larvae per minute.

2.5. Data analysis

To determine the effect of temperature (fixed factor) and pH (fixed factor) on fertilization success, a factorial analysis of variance (ANOVA) was used. To assess the effect of time (fixed factor), temperature (fixed factor), pH (fixed factor) and tank (random factor) on larval growth (total body length, rod length, body width, and the asymmetry between rods), factorial ANOVAs Model III (i.e. mixed model) were used. To describe larval development and how it differed among treatments, descriptive statistics of the proportion of larvae in each development stage in each treatment over time were applied. To determine if the effects of OAW on growth were solely due to differences in larval development rate, we compared the size (total body length, rod length, body width, and the asymmetry between rods) of four-arm pluteus larvae (present from days 1 to 13) between treatments, regardless of their age, using one-way ANOVAs, or if the data was non-parametric (i.e. was not normal and/or variances were not homogenous), Kruskal-Wallis tests. When significant differences were found, Tukey post-hoc multiple comparison tests or equivalent non-parametric tests were performed to compare all treatments. To assess the effect of temperature (fixed factor) on respiration rate, a two independent samples *t*-test was used. The statistical software R was used to conduct all statistical analysis.

3. Results

3.1. Experiment 1: fertilization success

In trial 1, of the 50 sea urchins collected, nine males and nine females spawned. The initial egg and sperm stock concentrations were 6441 eggs mL^{-1} and 11.791 × 10^6 cells mL^{-1}, respectively. The egg and sperm stocks were pooled, the water level was adjusted and the final ratio was 1847 sperm cells:1 egg. Fertilization success was significantly affected by temperature ($p = 3.4 \times 10^{-5}$) but was not significantly affected by pH ($p = 0.11$). Specifically, fertilization success was much lower at the higher temperature (31 °C, OW and OAW treatments) than at current temperature (28 °C, ambient and OA treatments, Fig. 2): 88.4 ± 1.4%, 88.4 ± 1.4%, 72.4 ± 5.5%, and 81.5 ± 1.7%, respectively for ambient, OA, OW and OAW treatments. The interaction between temperature and pH was not significant ($p = 0.12$).

In trial 2, of the 50 sea urchins collected, 13 males and nine females spawned. Egg and sperm stock concentrations were 3821 eggs mL^{-1} and 29.031 × 10^6 cells mL^{-1}, respectively. The egg and sperm stocks were pooled, the water level was adjusted, and the final ratio was 7896 sperm cells:1 egg, a higher sperm to egg ratio. Fertilization success rates were higher in Trial 2 than in Trial 1 (Fig. 2): 97.8 ± 0.3%, 95.4 ± 1.7%, 97.9 ± 0.7%, 97.0 ± 1.8%, respectively for ambient, OA, OW and OAW treatments. In this trial, fertilization success was not significantly affected either by temperature ($p = 0.84$), pH ($p = 0.49$), or the interaction between the two ($p = 0.29$).

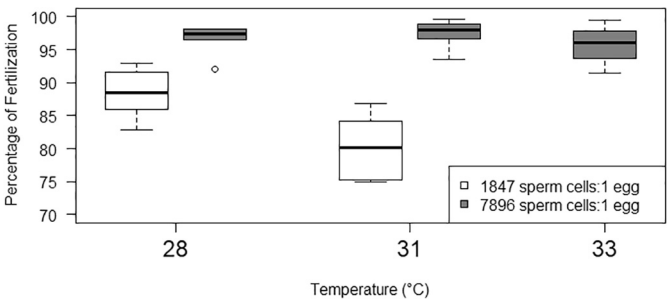

Fig. 2. Fertilization success under lower sperm: egg ratio (Trials 1, white) and higher sperm: egg ratio (Trial 2, grey) for each temperature ($n = 3$ replicate jars per treatment, with 2 subsamples per jar) (note: since pH did not have a significant effect on fertilization success, the data was pooled).

3.2. Experiment 2: larval growth and development

Data for larval growth and development was collected up to day 13. During this period, larvae went through multiple larval stages (blastula, gastrula, prism, early pluteus, 4-arm pluteus, 6-arm pluteus, and 8-arm pluteus, Fig. 3). The total body length of larvae increased significantly over time ($p < 2.2 \times 10^{-16}$, Fig. 4A). Larvae reared under lower pH conditions (OA and OAW treatments) had significantly smaller total body length than larvae reared under current pH conditions (ambient and OW treatments) ($p = 4.51 \times 10^{-5}$, Fig. 4A). Total body length was not significantly affected by temperature ($p = 0.33$) nor it differed between tanks ($p = 0.81$). None of the interactions between time, temperature, pH and tanks were significant (all $p > 0.05$).

Average rod length of larvae increased significantly over time ($p < 2.2 \times 10^{-16}$, Fig. 4B), but it was smaller in larvae reared under decreased pH conditions (OA and OAW) than in those reared under current pH conditions (ambient and OW) ($p = 5.75 \times 10^{-6}$, Fig. 4B). Average rod length was not significantly affected by temperature ($p = 0.63$, Fig. 4B). Tank and all interactions between time, temperature, pH and tanks were not significant ($p > 0.05$).

Total body width of *L. variegatus* larvae increased significantly over time ($p = 1.75 \times 10^{-7}$, Fig. 4C). Temperature and pH did not significantly affected total body with ($p = 0.54$ and $p = 0.73$, respectively; Fig. 4C). Tank and all interactions between time, temperature, pH and tanks were not significant ($p > 0.05$).

Asymmetry between rods in L. *variegatus* larvae increased significantly with decreased pH ($p = 1.29 \times 10^{-3}$), but not over time ($p = 0.05$) nor with temperature ($p = 0.16$) (Fig. 4D). Specifically, larvae reared under low pH (OA and OAW treatments) were significantly more asymmetric than larvae reared under current pH (ambient and OW treatments, Fig. 4D). Tank and all interactions between time, temperature, pH and tank were not significant ($p > 0.05$).

At 28 °C and pH 7.8 (OA), larval development (complexity) of *L. variegatus* was delayed, as reflected by the greater variation in development 1-day post-fertilization, and because this treatment had more larvae in earlier stages of development (blastula) than the ambient treatment (Fig. 5). At 31 °C (OW and OAW treatments), larvae exhibited minor delays in development on day 2 (more larvae in the gastrula and prism stages) relative to the ambient treatment; however, starting on day 5, larvae in the warmer treatments were more developed (some already had 6 arms) relative to ambient (which only had 6-arm pluteus starting on day 6, Fig. 5), suggesting that increased temperature accelerated development. Development did not differ substantially between OW and OAW treatments (Fig. 5). Thus, increased temperature appears to outweigh the negative effects of pH on development.

The effects of OAW on growth (size) were not due to differences in larval development rate (complexity) because, regardless of their age, the total body length and average rod length of 4-arm pluteus larvae also differed significantly between treatments ($p = 4.8 \times 10^{-10}$ and 4.9×10^{-12}, respectively). Four-arm pluteus larvae reared in the OW treatment had a slightly, but significantly, smaller average rod length than larvae reared in ambient conditions. Four-arm pluteus larvae reared in the OA treatment had a significantly smaller total body length and average rod length than larvae reared in ambient conditions. The 4-arm pluteus larvae reared in OAW conditions were not significantly different from the larvae reared in the OA treatment, and had a significantly smaller total body length and average rod length than larvae reared at ambient conditions; their total body length was not significantly different from larvae reared in the OW treatment, but their average rod length was significantly smaller; their total body length and average rod length was significantly smaller. The total body width and

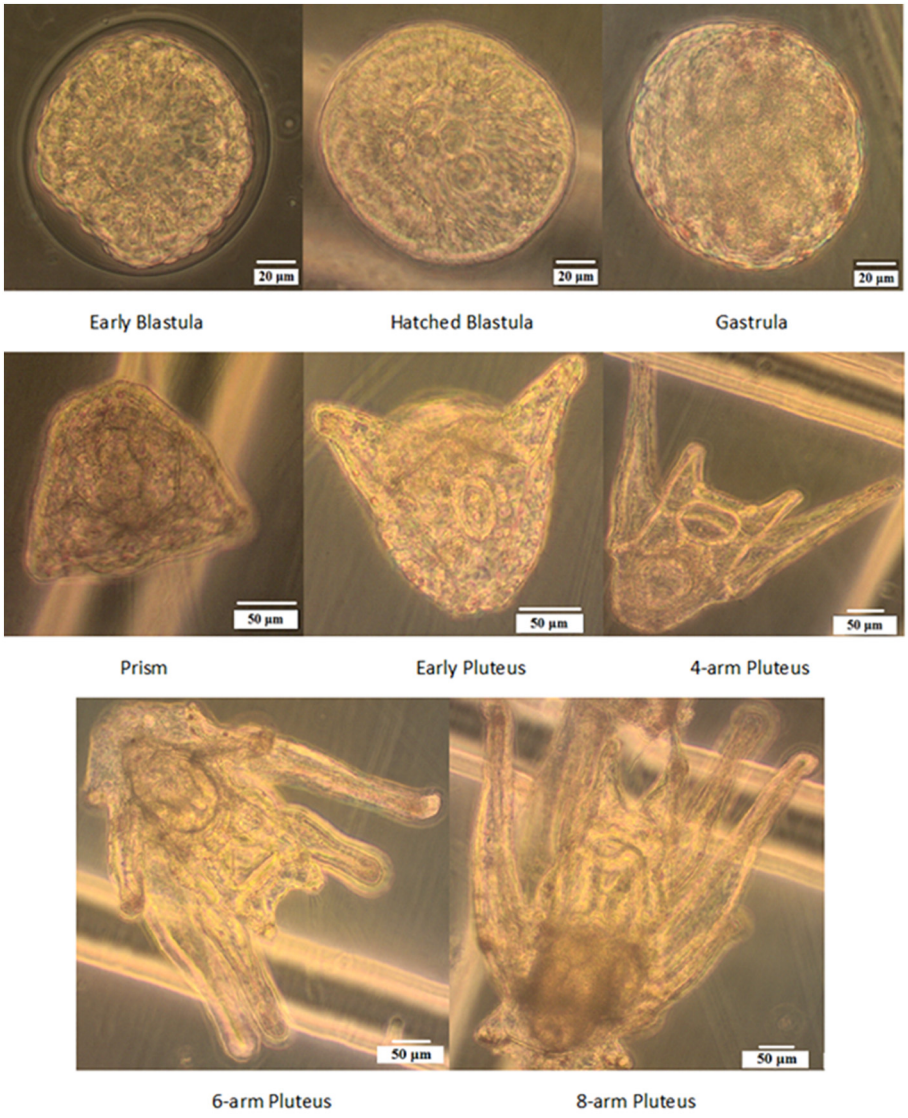

Fig. 3. Developmental stages of *L. variegatus* echinopluteus larvae under ambient conditions.

asymmetry of 4-arm plutei did not significantly differ between treatments ($p = 0.28$ and 0.06, respectively).

3.3. Experiment 3: larval respiration

Lytechinus variegatus larvae consumed significantly more oxygen at 31 °C ($2.17 \times 10^{-4} \pm 2.74 \times 10^{-5}$% O_2·larva^{-1}·min^{-1}) than at 28 °C ($9.45 \times 10^{-5} \pm 2.17 \times 10^{-5}$%$O_2$·larva^{-1}·min^{-1}, $p = 0.02$, Fig. 6).

4. Discussion

Lowered pH (OA) had no effect on fertilization success of *Lytechinus variegatus* but delayed larval development, stunted growth, and caused larvae to become more asymmetric. Elevated temperature (OW) decreased fertilization success at a lower sperm to egg ratio (1847:1) and accelerated larval development but had no significant effect on larval growth. Under both warming and acidification (OAW), fertilization success was reduced, larval growth stunted, and larvae became more asymmetric, indicating that the stressors have additive effects. Although larvae developed more rapidly under OAW conditions, they remained smaller than the larvae reared at ambient conditions, suggesting that the negative effects of reduced pH were outweighed by the accelerated development under increased temperature (all results are summarized in Table 1).

An end-of-the-century pH (7.8) did not reduce fertilization success of *L. variegatus*, most likely because this value is still within the daily range this species experiences on its natural environment. Driven by photosynthesis and respiration, the pH of seawater in coral reef ecosystems fluctuates throughout the day and is typically lower before dawn and higher during early evening (Hofmann et al., 2011). The anticipated pH changes resulting from ocean acidification projected for surface ocean waters by 2100 are similar to the ones registered in many locations where *L. variegatus* occurs. For example, *L. variegatus* occurs in Tampa Bay, where pH currently averages 8.1 but declines to as low as 7.95 in mid-afternoon when photosynthesis peaks (Yates et al., 2007). Heavy rains can expose this coastal species to even lower pH levels, particularly near inlets. Previous studies have found that low levels of pH can inhibit the acrosomal reaction of sea urchin sperm (Gregg and Metz, 1976) and lower sperm motility and respiration hindering internal ATPase activity (Christen et al., 1983). Reduced sperm motility can in turn decrease fertilization success, as reported in several sea urchin species (Kurihara and Shirayama, 2004; Havenhand et al., 2008; Moulin et al., 2011; Kapsenberg et al., 2017). However, such negative effects of pH on fertilization success in sea urchins have only been reported for pH levels ranging from 6.8 to 7.7, which represent scenarios projected for approximately the years 2150–2300+ (Caldeira and

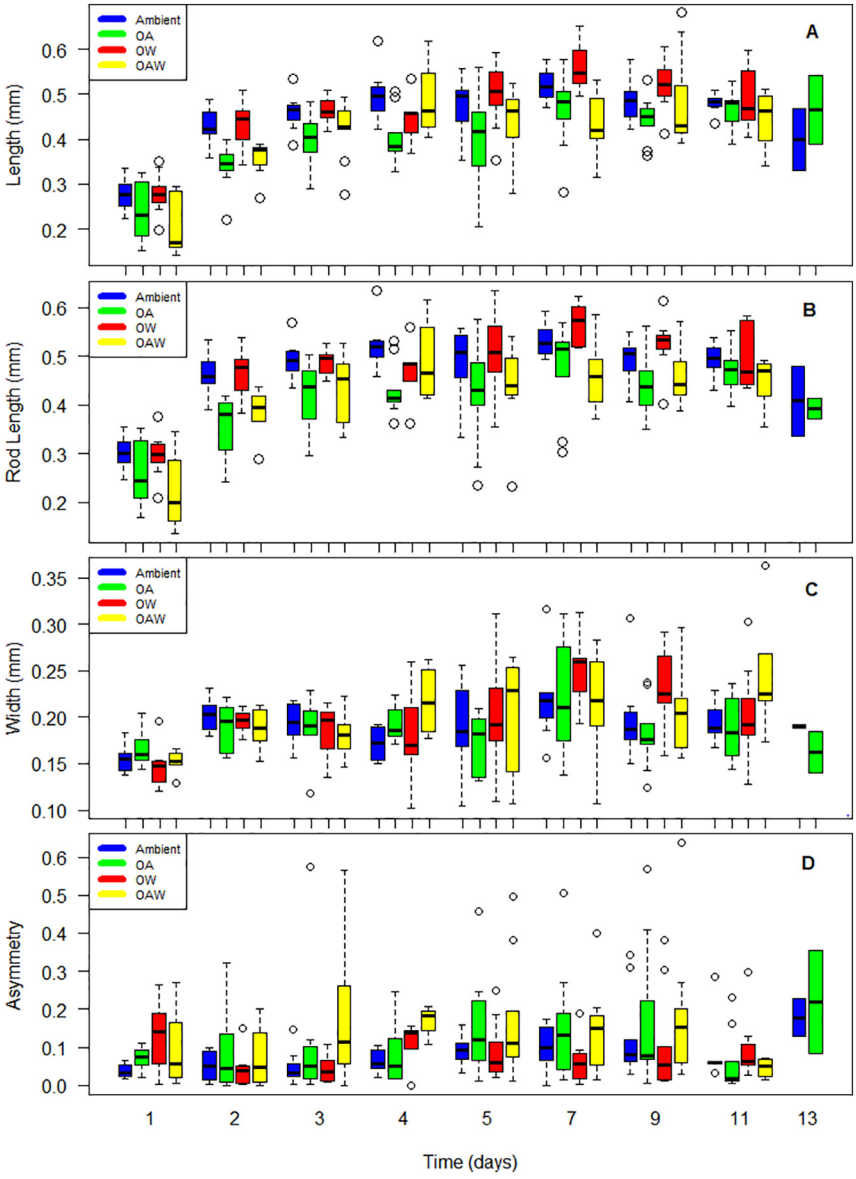

Fig. 4. Larval measurements, specifically (A) total body length (mm), (B) average rod length (mm) (C) total body width (mm), and (D) larval asymmetry, in each treatment over time (days) ($n \geq 10$ per treatment/day).

Wickett, 2003). In this study, we decided to only test the effects of pH for a near-future (end of the century) scenario because projecting beyond that point may overlook the potential for acclimation or even adaptation.

Increased temperature decreased fertilization success only when the sperm to egg ratio was lower, likely because higher temperatures reduce sperm longevity (time during which the sperm remains motile; Alavi and Cosson, 2005) but this is only problematic when the chances of encounter between sperm and egg are lower. Fertilization success is typically greater under higher sperm to egg ratios because it increases the chances of encounter between egg and sperm (Levitan et al., 1992; Byrne et al., 2010b). At lower sperm to egg ratios, the frequency of encounter between egg and sperm is generally lower, and thus fertilization success is more dependent on sperm velocity and longevity (Levitan et al., 1992; Levitan, 2000). Warmer temperatures tend to increase sperm velocity and thereby might boost fertilization success (Mita et al., 1984; Alavi and Cosson, 2005; Dadras et al., 2017). However, thermal stress also increases sperm's metabolic demand, shortening sperm longevity, and potentially compromising fertilization success (Levitan, 2000; Alavi and Cosson, 2005; Dadras et al., 2017). In this study, the reduced fertilization success with lower sperm to egg ratio under warmer conditions is likely explained by the reduced sperm longevity. Specifically, while the sperm may have swam faster under warmer conditions, it likely fatigued or died before encountering an egg, resulting in a lower fertilization rate. The decreased longevity under warm conditions did not affect fertilization success in the second trial because the sperm to egg ratio was much higher, and thus the sperm quickly found the eggs. There was no evidence of reduced fertilization from polyspermy because fertilization was highest when the sperm to egg ratio was also higher; however, these embryos were not monitored for development, thus we cannot be completely certain.

Warmer temperatures increased larval respiration (a proxy for metabolism, Fig. 6) and larval developmental rate (Fig. 5) in *L. variegatus*, outweighing the negative effects of reduced pH on larval development and skeletogenesis. As in other sea urchin species (Sheppard Brennand et al., 2010; Stumpp et al., 2011; Byrne et al., 2013a), reduced pH slowed larval development in *L. variegatus* (Fig. 5), likely because of down-regulation of genes central to energy metabolism and biomineralization (O'Donnell et al., 2010). However, the positive effect of temperature counteracted this negative effect of reduced pH on metabolism

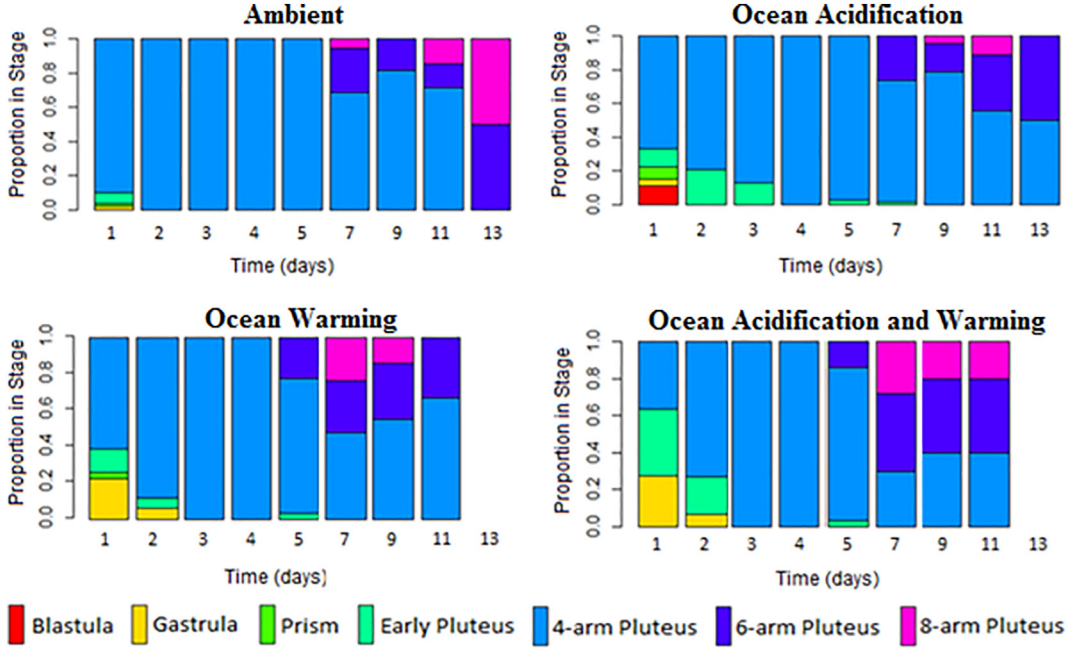

Fig. 5. Larval Development of *L. variegatus* displayed by proportion of larvae in each stage over time (days) for each treatment (n ≥ 10 per treatment/day).

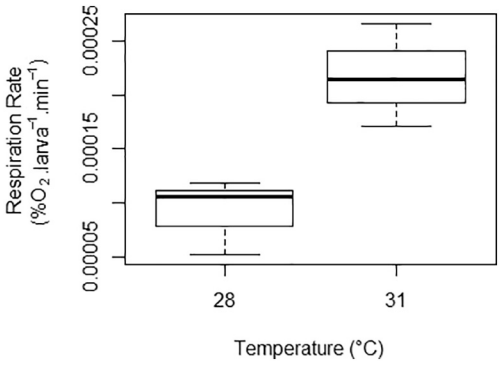

Fig. 6. Respiration of *L. variegatus* larvae (% O_2·larva^{-1}·min^{-1}) at ambient and warm temperature (n = 3 per treatment).

Table 1
Summary of effects of acidification and/or warming on the early development of *Lytechinus variegatus* (↑ increase, ↓ decrease, 0 no effect, – not tested).

Measurement	Condition		
	Acidification (pH 7.8, 28 °C)	Warming (pH 8.1, 31 °C)	Warming and Acidification (pH 7.8, 31 °C)
Fertilization	0	↓	↓
Total body length	↓	0	↓
Average rod length	↓	0	↓
Asymmetry	↑	0	↑
Total body width	0	↑	↑
Larval development	↓	↑	↑
Respiration	–	↑	–

and larval development rate (Fig. 5). At warmer temperatures, *L. variegatus* larvae consumed nearly twice as much oxygen than those at ambient temperature (Fig. 6), reflecting an accelerated metabolism. Warm temperatures increase enzymatic activity, which in turn, accelerates physiological processes, such as metabolism and cell division (Savage et al., 2004). As observed in other sea urchin species (Sheppard Brennand et al., 2010; Byrne et al., 2013a), faster metabolism (Fig. 6) and rates of cell division caused by warmer temperatures led to faster larval development, i.e., a quicker progression to more complex larval stages (Fig. 5). However, increases in metabolic rates decrease the scope for growth (Stumpp et al., 2011) because larvae typically allocate energy primarily to survival, then to development (complexity), and only then to growth (size). When larval metabolism increases, larvae may consume energy reserves more quickly than they can replenish them through feeding. Thus, while the larvae *L. variegatus* developed faster under warmer conditions, this increase in complexity was not accompanied by a faster growth.

Elevated temperature did not affect total body length, average rod length, total body width and asymmetry of *L. variegatus* larvae, but reduced pH decreased larval length and increased asymmetry likely due to the diversion of energy from development and growth to homeostasis. Under decreased pH, calcium carbonate in seawater is less available for uptake because free carbonate ions tend to form bicarbonate ions (Fabry et al., 2008; Doney et al., 2009). Sea urchin larvae have internal skeletons and, to some extent, have the ability to maintain the pH within their cells at higher levels suitable for calcification/skeletogenesis (Stumpp et al., 2012). However, keeping pH homeostasis has energetic costs (Jager et al., 2016), leading to modifications in energy allocation and thus slower development and growth under OA (O'Donnell et al., 2010; Stumpp et al., 2011). This developmental delay may be also due to the effects of ocean acidification on the expression of genes related to skeletogenesis in sea urchins. Specifically, genes central to energy metabolism and biomineralization are downregulated in larvae of *L. variegatus* and other sea urchins reared in a lower pH (O'Donnell et al., 2010; Stumpp et al., 2011; Raven et al., 2005; Kroeker et al., 2010). Decreased pH also caused an increase in asymmetry in L. *variegatus* (this study) and other sea urchin larvae (Byrne et al., 2013a; Uthicke et al., 2013; Lamare et al., 2016). Under low pH, larvae allocated the available calcium carbonate ions disproportionally between rods leading one rod to grow more than the other. The mechanism that regulates this differential allocation of carbon ions to the rods remains to be studied. Larval asymmetry may compromise a larva's ability to feed and control movement and placement in the water column (Strathmann, 1975). The prevalence of rod asymmetry and decreased growth in sea urchin larvae due to elevated acidity suggests that ocean acidification will impair the ability of L.

variegatus larvae to develop, grow, and produce a normal, symmetric larval skeleton that ultimately compromises their survival, dispersal, and recruitment.

Species' tolerance for changes in environmental conditions depends in part on the magnitude of daily/annual fluctuations in the region in which they evolved; however, this is not always certain. Here, we studied the fertilization, larval development and growth under future OAW conditions of a sea urchin species from tropical/subtropical waters. We found that temperature affected larval development more than pH, although larval growth decreased under low pH, but was not affected by warmer temperature. The combined effects of warming and acidification on sea urchin larvae have only been studied in fewer than ten species (Byrne et al., 2009; Sheppard Brennand et al., 2010; Foo et al., 2012; Byrne et al., 2013a; Byrne et al., 2013c), hence it is hard to reach conclusions on the relative susceptibility of species to OAW based on differences in magnitude of annual temperature and pH variations between latitudes or proximity to coastlines where they evolved. In some animal groups (e.g. terrestrial ectotherms such as lizards, Tewksbury et al., 2008), it has been hypothesized and documented that, because temperate species have a wider temperature tolerance range, they should be more tolerant to ocean warming than tropical species. So far, the results of this and previous studies in sea urchin larvae seem to point to the opposite: higher tolerance to warming in tropical sea urchin species relative to temperate species. The larvae of a tropical species, *Tripneustes gratilla*, grew faster as a result of a 3 °C increase (Sheppard Brennand et al., 2010). In addition, larval growth was recorded as optimal between 27 and 34 °C in the tropical sea urchin *Echinometra lucunter*, with the maximum at +6 °C above ambient temperature (Sewell and Young, 1999). By contrast, a temperature increase of 4–6 °C above maximum ambient decreased growth and development in larvae of two temperate species (Fujisawa, 1989; Byrne et al., 2009). Tropical regions experience narrower sea surface temperature fluctuations, but values remain at the high end of the spectrum year-round. In temperate regions, temperatures vary much more widely throughout the year but reach high levels only during a much shorter period. This difference in duration of annual exposure to higher temperatures may explain why tropical sea urchins may be more tolerant to increases in temperature than temperate species.

Warming and acidification are affecting the early life stages of many different marine organisms. For sea urchins, the combined effects of warming and acidification reduced fertilization success, accelerated larval development, reduced growth and increased asymmetry. Smaller and asymmetric larvae will be less able to properly swim, feed, or control their position in the water column (Clay and Grunbaum, 2011). The results of this study suggest that *L. variegatus* will experience reduced fertilization success, suffer greater larval mortality, disperse less and have a harder time to settle on a suitable environment. These deleterious effects are likely to have severe impacts on the its persistence, because they affect rates of connectivity and gene flow between populations and thus rates of recovery after disturbances. Assessing the impacts of OAW on both adult and larval stages of key ecosystem species, such as sea urchins, are critical for accurately predicting the future of marine ecosystems.

Acknowledgements

This work was supported by NSU President's Faculty Research and Development Grant # 335412. *Lytechinus variegatus* were collected under Florida Fish and Wildlife Conservation Commission permit #SAL-17-1902-SRP. We thank Dr. Ana I. Catarino for insightful discussions, Dr. Charles Messing for edits to earlier drafts of this manuscript, and members of the Marine Larval Ecology Lab that assisted with this project: A. Anderson, A. Galarno, H. De Marchis, T. Kamerman, S. King, S. Koerner, A. Kuba, N. McMahon, A. Nease, J. Robbins and M. Stephenson.

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